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978-3-330-05069-3

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**Impressum / Imprint**
Bibliografische Information der Deutschen Nationalbibliothek: Die Deutsche Nationalbibliothek verzeichnet diese Publikation in der Deutschen Nationalbibliografie; detaillierte bibliografische Daten sind im Internet über http://dnb.d-nb.de abrufbar.


Bibliographic information published by the Deutsche Nationalbibliothek: The Deutsche Nationalbibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data are available in the Internet at http://dnb.d-nb.de.


Coverbild / Cover image: www.ingimage.com

Verlag / Publisher:
LAP LAMBERT Academic Publishing
ist ein Imprint der / is a trademark of
OmniScriptum GmbH & Co. KG
Bahnhofstraße 28, 66111 Saarbrücken, Deutschland / Germany
Email: info@omniscriptum.com

Herstellung: siehe letzte Seite /
Printed at: see last page
**ISBN: 978-3-330-05069-3**

Zugl. / Approved by: TRAKYA UNIVERSITY STARA ZAGORA FACULTY OF AGRICULTURE, Bulgaria

## ABBREVIATIONS USED IN THE TEXT

**ALT** (alanine aminotransferase ) - a liver enzyme
**AST** (aspartate aminotransferase) - a liver enzyme
**BUN** – blood urea nitrogen
**MUN** – milk urea nitrogen
**COD** – ovarian cysts
**CLP** – persisting yellow body
**P4** – progesterone

This dissertation thesis is going to be defended on 13.02. 2015 at 10.00 o'clock at the University of Trakya in Stara Zagora. Once the defending process is complete, these materials will be at a disposal at the Research Department of the Agricultural faculty and will be available on the University website www.uni-sz.bg.

**Note:** The numbering of tables, figures and photographs used in this summary is the same as used in the dissertation.

## INTRODUCTION

The reproductive problems of high-productive dairy herds represent one global multifactor issue which requires a multidisciplinary approach. The selection of maximal dairy productivity is related to the selection according to some other characteristics such as body condition and increased risk of occurrence of metabolic and reproductive problems of dairy cow breeds. The reproductive system types of dysfunction such as: ovarian cysts, ovarian hypo-function, delayed or absent ovulation etc. are directly caused by metabolic disorders in the body.

The metabolic profile is a significant marker of physiological and reproductive status and serves as a diagnosis of many subclinical diseases. In highly productive cows, measuring the concentration of urea nitrogen in blood and milk provides additional information related to the energy and protein absorption. Preparation and screening samples of milk is far more accessible method than the invasive method for sampling blood, because most researchers suggest routine methods for determining the effectiveness of protein diet while determining the concentration of urea nitrogen and other metabolites in milk samples. Optimization of protein energy balance rations will improve the reproductive and productive performance of dairy cows and physicochemical and technological properties of milk.

## PURPOSE AND OBJECTIVES

The **purpose** of this dissertation is to determine the reproductive problems, metabolic profiling and established relationship between the dysfunction of the reproductive system and the registered blood-serum indicators as well as their impact on the physical-chemical and technological properties of milk in the Holstein-Frisian cows.

In order to solve this problem we have set the following **tasks:**

1. **Conducting a two-year research of the forms and causes of infertility in dairy farm**

   **1.1.** Grouping and seasons' analysis

2. **Biochemical analysis of blood samples obtained from problematic cows in the period of reproduction**

**2.1.** Determining the serum levels of macronutrients: calcium (Ca), phosphorus (P), potassium (K), sodium (Na) and chloride

**2.2.** Determining the serum levels of: general protein, urea, liver enzymes (AST and ALT), progesterone and glucose

**2.3.** Grouping and seasons' analysis

**3. Analysis of the depending relations between some serum metabolites and excretion into milk secretion in cows with reproductive pathology and cows with normal reproduction**

**3.1**. Study of the depending relations between metabolites in cows with proven reproductive disorder

**3.2**. Study of the depending relations between metabolites in cows inseminated with no problems while the first insemination

**3.3.** Benchmarking

**4. Laboratory analysis of milk samples and determining the depending relations between the studied metabolites and excretion into the milk secretion**

**4.1.** Laboratory milk testing of cows with normal values of metabolites

**4.2**. Laboratory milk testing of cows with elevated values of metabolites

**4.3.** Laboratory testing of the coagulation capacity of milk from cows with different values of metabolites

## MATERIALS AND METHODS

The research was conducted in the period between the beginning of 2012 till the beginning of 2014. The studied cows belonged to a Holstein Frisian breed. The average age of the analyzed animals was 59-62 months. The dairy farm was located in the Pelagonia Region – Republic of Macedonia.

### 1. STUDY OF COWS WITH REPRODUCTIVE PROBLEMS

Within the experimental group with impaired reproduction there were included all cows with no signs of oestrus 45 days after calving (**Deletang et al., 1983).** Each cow with reproductive disorder has been examined according the following order:

1. Appearance
2. Vaginal examination with vaginoscope
3. Rectal examination of the reproductive organs
4. Ultrasound examination (ultrasound, Aloka 500 - 5 MHz linear transducer)

After the examination, the cows have been allocated into the following groups of animals with reproductive disorders:

- ovarian cysts
- ovarian hypo-function,
- presence of persisting yellow body(corpus luteum persistence).

After the diagnosis, venous blood was obtained in vacuum tubes (Vacuumtainers). ***In cows, whose problems persisted for a longer period (several months) in order to monitor their status, blood was being repeatedly obtained.***

The experiment was conducted over 148 cows. Starting from the initially studied and included animals whose blood was obtained and examined, there were 41 of them who showed sings of oestrus within the period of 15 days and thus they were inseminated. On the day of artificial insemination, blood samples for laboratory analysis were obtained again. After a period of 40 days, while the ultrasound examination, there were 38 cows with confirmed pregnancy. The results of those samples were applied in a comparative analysis which led to formation of a new group ***"cows with normal reproductive status".***

***Studied group – first assignment:*** 148 cows – 40 (spring season), 29 (summer season), 31 (autumn season) and 48 (winter season).
***Analyzed blood samples – second assignment:*** Calcium, Phosphorus, Sodium, Potassium and chlorides.
***Analyzed blood samples – second assignment:*** general proteins in blood, BUN, AST, ALT, progesterone – P4 and glucose.
***Analyzed blood samples – third assignment:*** BUN, AST, and progesterone – P4. The cows were divided in two groups: 1) cows inseminated for the first time with confirmed pregnancy after insemination – 38, and 2) animals with reproductive disorders - 110. The second group was divided in two subgroups depending on the urea content in the blood. The first subgroup „A" consisted of 49 cows with low urea content value (2-6 mmol/L) and the second subgroup „B" consisted of non-pregnant animals with high urea content value (over 6 mmol/L) – 61.
***Analyzed milk samples – fourth assignment:*** milk samples of cows with normal urea level in milk – 10 and high urea level – 15.

## 2. LABORATORY ANALYSES OF BLOOD

The blood samples for biochemical analysis of the selected cows with reproductive disorders have been obtained on a weekly basis during the experimental period. The manipulation was administered 2-3 hours after the morning meal. The blood was obtained by punctuation in *vena coccygea* in vacuum tubes (CE, ISO 13485), an was transported with a removable refrigerator to a biochemical analysis laboratory (Oraldent – city of Bitola). The blood serum for biochemical analysis was obtained after centrifuging the sample at 3000 rpm / min. for 5 minutes.

The experiment to the blood samples was conducted according the following methods:

- Photometric method (CPC) to determine the level of Calcium **(Barnett R. N. et al., 1973)**
- Photometric UV method to determine the level of Phosphorus **(Gamst, et al., 1980)**
- Photometric turbo-dynamic method to determine the level of Potassium **(Tietz, 2006)**
- Colorimetric method to determine the level of Sodium **(Henry et. al., 1974)**
- Colorimetric method to determine the level of chlorides **(Tietz, 1995)**
- Enzymatic colorimetric method to determine the level of glucose **(Tietz, 1995)**
- Photometric method to determine the level of general proteins **(Weichselbaum, 1946)**
- Photometric enzymatic method to determine the level of urea **(modified reaction of Berthelot, 1859)**
- Method to determine Aspartat Aminotransferase – AST **(Thefeld, 1974)**
- Method to determine Alanine Aminotransferase - ALT **(Thefeld, 1974)**
- Enzymatic chemiluminescent immunochromatographic method to determine the level of progesterone **(Aufrère and Benson, 1976)**

## 3. LABORATORY ANALYSES OF MILK

The milk samples for analysis were obtained during the morning milking of cows according the rules for providing milk samples, by placing them in an individual sterile plastic cup (50 ml) and placing it into the individual collectors of the milking systems. The individual milk samples (without addition of preservatives) have been transported by a mobile refrigerator to an independent laboratory ("Mlekokontrol – Pelagonija"- city of Bitola) in order to be analyzed. The milk analysis was conducted within the period of three hours after milking.

The samples of raw cow's milk were submitted to the following analysis according to the reference methods of **IDF - ISO-AOAC** respectively:

- Chemical composition (proteins, milk fat, lactose) and dry non-fat residue – by high-infrared analyzer Lactoskope**,** Germany
- General number of somatic cells - by Somaskop, Alfa Laval, Germany
- pH (active acidity) - by pH meter model Lab 860, SCHOTT, Germany
- titratable acidity - according Sokslet Henkel - SH0 (modified method by Mores with using 0,1 mol/l sodium hydroxide for neutralizing 100 ml of milk)
- urea concentration – by Spectroquant Pharo 300, Merck, Germany – photometric method (Merck KGaA, 64271 Darmstadt, Germany)

**Laboratory testing of milk coagulation –** Testing of milk coagulation was conducted three hours after milking at the "Laboratory for chemical analysis and milk and milk products technology" (Faculty of Biotechnical Sciences, St. Kliment Ohridski – Bitola). Five liters of preheated milk on 35 °C of each examined cow were placed in a double bottom stabilizer, adding at the same time powder yeast (CHY–MAX POWDER EXTRA NB, CHR. HANSEN, the strength of the yeast being 2235 IMCU/g) according to the criteria of the manufacturer. The normal coagulation was

registered in the period between 45 to 60 minutes. Firstly, it was determined the period for initial coagulation and the period for final coagulation, as well as organoleptic characteristics of the coagulum. After dissection of the coagulum, the value of pH,$^0$SH and the amount of separated whey (synerezis) were determined.

**Table № 3.** Indicators for organoleptic assessment of curd

| Class | Appearance and curd characteristics |
|---|---|
| **I** | The curd is very well and firm. When kneaded it doesn't leave marks on the palm. The whey is well separated and is of typical yellow-green color. |
| **II** | The curd is well and not so firm. The whey is well separated and is of clear yellow-green color. |
| **III** | The curd is loose, soft and it doesn't stay firm. When kneaded it leaves marks on the palm. The whey is not well separated and is of white-green color. |
| **IV** | The curd is loose and it doesn't stay firm. It can not be cut with a knife and when kneaded it leaves marks on the palm. The whey is not well separated and is of milky white color. |
| **V** | The casein coagulation is minimal or lacking. The whey is of milky white color. |

## 4. STATISTICAL DATA ANALYSIS

In order to perform biometric data analysis, we have used the statistical package "STATISTICA" v. 6.1 (**StatSoft Inc., 2002**).

Calculating the average and standard deviation (mean ± SD) was conducted by the program module DESCRIPTIVE ANALYSIS.

By applying the module BASIC STATISTICS AND TABLES – one-factor analysis ANOVA we have established a statistical significance of differences between the mean values compared by LDS-test with confidence level of $p<0.05$.

In order to calculate the correlations between the studied parameters we have used CORRELATION program module at a level of significance $p<0.05$.

## RESULTS AND DISCUSSION

### 1. INVESTIGATION OF THE REPRODUCTIVE STATUS OF THE ANALYZED DAIRY FARM

After the analysis of infertility in the analyzed dairy farm a significant deviation from the norms was being registered. Of the basic reproductive disorders during the two-years period **(fig. № 1)** the percentage of cows with ovarian cysts is the highest **(*COD*)** (60.54%) after which comes the ovarian hypo-function (30.61%) and finally there occurs the persisting yellow body (8.84%).

***Because of the already established basic problem (COD) referring to infertility of the tested farm, the analysis of the results would cover only these values.***

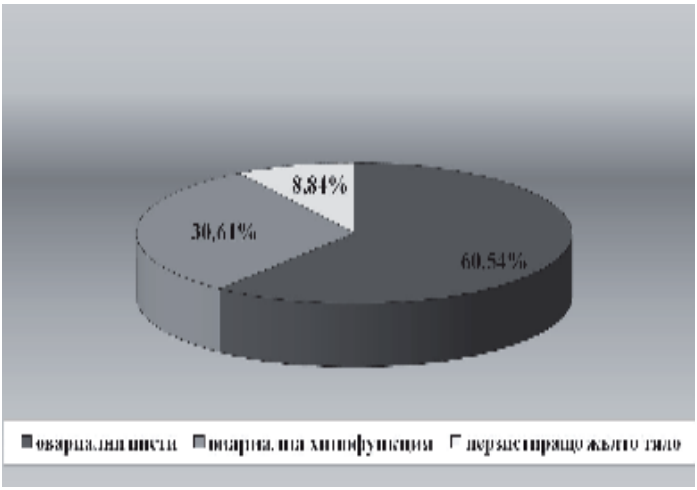



**Figure № 1.** Distribution of reproductive disorders in cows during the survey period

From the results presented in **table № 4** we have determined that for the period of one year there were diagnosed and analyzed 148 cows with reproductive problems occurring in the after-birth period (45-50 days after calving) which presented 43.23% of the whole herd. The ovarian cysts diagnosis varied from 48% to 76.6% during different seasons, where as the percentage of all clinical manifestations of reproductive disorders was between 19.73% to 31.97%. Some authors **(Nanda, 1989)** report cases of ovarian cysts in 6% to 19% of the cows. In our study, however, this percentage was significantly higher. The ovarian cysts of highly productive milk cows are significant

pathology of the ovaries as well as one of the main causes for infertility in contemporary dairy cattle breeding. (**Vanholder et al., 2006**).

**Table № 4.** Distribution of reproductive disorders in cows during the different seasons of the year

| Season of the year | Cows with reproductive disorders (nr.) | (%) | Persisting yellow body (%) | Ovarian cysts (%) | Ovarian hypo-function (%) |
|---|---|---|---|---|---|
| Spring | 40 | 27.22 | 12.50 | 52.50 | 35.00 |
| Summer | 29 | 19.73 | 0 | 48.28 | 51.72 |
| Autumn | 31 | 21.08 | 6.45 | 58.06 | 35.48 |
| Winter | 48 | 31.97 | 12.77 | 76.60 | 10.64 |

During the early after-birth period the cases of ovarian cysts are probably more frequent and 60% of cows who develop such kind of cysts before first ovulation, spontaneously recover their reproductive cycle **(Silvia et al.,2002).** The etiology and pathogenesis of this problem haven't been cleared yet. It mainly focus on the endocrine disorders in the hypothalamic - pituitary axis. There is evidence **(Grado-Ahuir et al., 2011)** that shows genetic predisposition of cows.

The seasonal occurrence of the reproductive disorders in milking cows of the analyzed farm is presented on **fig. № 2**. Of the total number of cows in the farm during the survey period, the "ovarian cysts" have been diagnosed in 60% of cows. The highest percent of animals with problems in different season was diagnosed during the winter – 76.6%, where as the lowest percent was diagnosed during the summer - 48.28%. There has been uptrend of morbidity in the period between June to December.

The **fig. №2** represents the dynamics of the diagnosed ovarian cysts, persisting yellow body and ovarian hypo-function in different seasons. The mathematically calculated curve presenting the cases of cysts is different to that of the general morbidity and hypo-function. While in most months examined, the percent of ovarian cysts is from 48% to 58% of the reproductive disorders (differences are statistically unreliable $p < 0.05$), in the months of December and January we found a significant increase ( 76.6% ) as in this period when the differences are statistically significant.

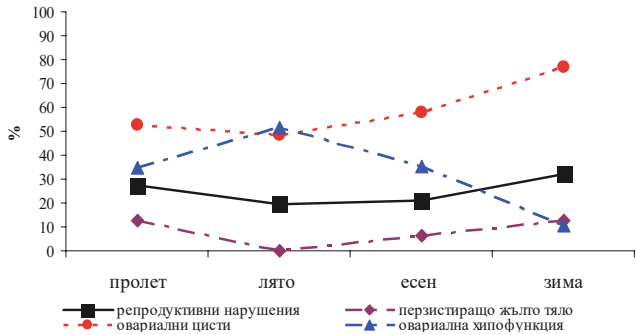
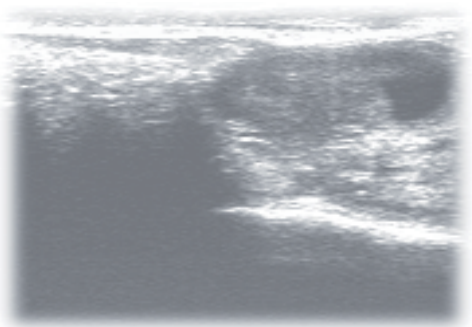


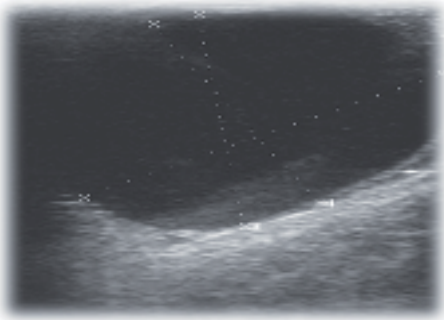

**Fig № 2.** Distribution of reproductive disorders of cows in different seasons of the year

Depending on the degree of luteinization, the ovarian cysts can be categorized as luteal and follicular. During our analysis, follicular cysts were more frequent than the luteal **(pic. № 2 и 3)**. This is in line with the research of **Zemjanis (1970),** who reports that only 30.5% of 1.191 cystic ovaries contain luteal cysts. The luteal cysts are connected with anoestrus which makes impossible to differentiate the follicular from luteal cysts on the basis of the reproductive behavior. The luteal cysts have thicker wall than follicular which is difficult to distinguish with rectal palpation.

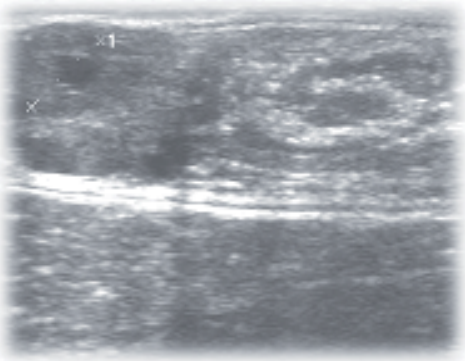
For that purpose we have conducted an additional ultrasound examination with a mobile ultrasound device. The pictures **№ 1, 2 and 3** present the ultrasound results for the identified ovarian cysts and the persisting yellow body. The ultrasound examination was a segment of the program for cows' reproductive status diagnostics.



**Picture № 1.** Ultrasound picture of a normal ovary with follicle (orig.)



**Picture № 2.** Large follicular cyst larger than 5 cm. (orig.)



**Picture № 3.** Persisting yellow body (orig.)

The obtained percentage of cows with persisting yellow body was 8,8%. This disorder mainly occurs under the influence of higher ammonia concentration in the follicular fluid. The ammonia is a metabolite of protein dissolution and its high serum levels can be reduced by balancing the physiological system of the cow. **Hammon et al. (2005**) observed negative changes in growth and metabolism of the granulose cells of the ovarian follicles in *in vitro* experiments after the addition of ammonia in non-physiological parameters

The third group of reproductive disorders **(fig. № 1)** established after the process of examination consists of the ovarian hypo-functions (30.61%), expresses by the lack of estrus (anestrus). The main causes for this disorder are the environmental conditions – insufficient or low-quality food, climatic conditions, lack of moving and presence of different contagious or other kinds of diseases. **(СкворединЕ.Н, 2009)**.

## 2. Biochemical analysis of the serum components

In this section we make a detailed analysis of some of the metabolites in the blood serum of the problematic reproductive cows alongside its other effects are separated and can be detected in milk secretion. Genital activity and fertilization of animals is the most sensitive indicator of their reaction to the environment and above all to nutrition. With longer adverse affect of the external environment there occur disturbances in the maturation and secretion of the whole eggs, and later permanent irreversible pathological changes in the ovaries.

The results of the biochemical analysis of the components; calcium, phosphorus, potassium, sodium and chloride derived from blood samples of problematic reproductive cows are presented in Table. № 5.

It is noteworthy that in most cows the levels of macronutrients are normal as it applies mainly to phosphorus and calcium (calcium 2.2-3.1 mmol / L; phosphorus 1.5-2.9 mmol / L). There are established differences in the seasons and the respective months. The lowest levels of calcium (2.61 ± 0.47 mmol / L) are found in blood samples of problematic cows in spring; March and April and the highest (3.12 ± 0.37 mmol / L) in the autumn. According to Lincoln et al. (1990) in the analysis we must consider and milk yield of cows, which in most cases is relatively high and has a strong influence on reproduction. In our research there is significant seasonality in the levels of macroelements, which has proven a reliable ($p < 0.05$) difference (Table . № 5).

When considering the effect of calcium and phosphorus on reproductive functions in cows, there should be considered not only the absolute content and ratio, but also the quantity of the ingredient per 100 grams of digestible protein in the ration. The highest fertility of cows (Goff, 2000) is registered in the presence of phosphorus from 3.5 - 3.9 grams per 100 grams of digestible crude protein in the diet. Lower values give a negative impact on reproduction.

### 2.1. Analysis of the metabolism of calcium and phosphorus

Our study shows a gradual reliable increase of the serum levels of calcium coming from spring to autumn and winter (Fig. № 3). Since the established values are within the normal limits, we can not seek a positive relationship only between calcium and reproductive diseases. This is observed as a process of comparable calcium level with other metabolites.

The adequate ratio of concentrations of calcium (Ca) and phosphorus (P) in the blood are vital for the normal function of animals. Physiological mechanisms for maintaining the serum Ca and P concentrations normal, effectively fulfill their role in much of the time of lactation **(Horst et al., 1994).** In some cases, these homeostatic mechanisms fail and there appear metabolic diseases. For normal homeostasis, calcium in the blood of adult cows should be maintained at about 8.5-10 mg / dL. This

means that there are about 3 g of calcium in the plasma of a cow of 600 kg., since the complete extracellular production is only 8-9 grams of calcium.

The absorption of calcium in the body is affected by the protein content in the rations. Some amino acids (arginine and lysine) facilitate its absorption, while fat and fiber lower it. Our results did not contain low levels, which show the absence of lowering the absorption factors. This is a consistent research of **Kincaid et al. (1981**). Dairy cows produce daily amount of colostrum or milk, which contains 20-30 g calcium. To prevent a reduction in blood calcium whose failure leads to a variety of serious consequences for life processes, the cow must replace calcium lost in the milk by removal of calcium from the bones or by increasing the effective absorption of feed **(Hove, 1986).**

A deficiency of calcium in cows, leads to more delayed involution of the uterus after birth, for these cows have difficulty in the birth process. Although we did not found a dramatic decrease in its levels, authentically the lowest levels are found in late winter and early spring. The lack of calcium leads to retention of the placenta and the relegation of the vagina and uterus. The ratio of Ca: P in the diet should be from 1.5: 1 to 2.5: 1 **(Kincaid et al., 1981).**

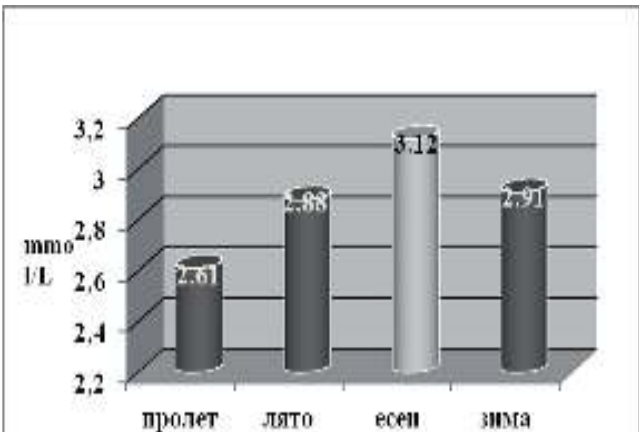



Figure № 3. Average serum calcium levels during different seasons

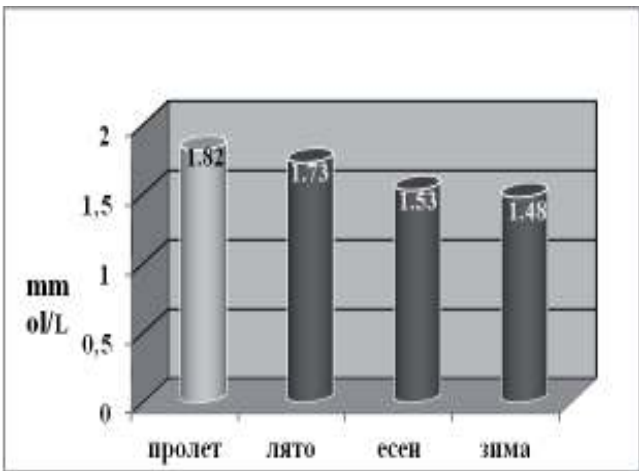



Figure № 4. Average serum phosphorus levels in different seasons

**Table № 5**. Contents of macro-elements in the blood plasma of cows during different seasons of the year **(mean ± SD)**

| Indicators | spring | | summer | | autumn | | winter | |
|---|---|---|---|---|---|---|---|---|
| | ref. | | ref. | | ref. | | ref. | |
| **Calcium**, mmol/L | 53 | 2.61 ± 0.47 $^{ABC}$ | 29 | 2.88 ± 0.37 $^{AD}$ | 34 | 3.12 ± 0.37 $^{BDE}$ | 32 | 2.91 ± 0.32 $^{CE}$ |
| **Phosphorus**, mmol/L | 53 | 1.82 ± 0.49 $^{AB}$ | 29 | 1.73 ± 0.44 $^{C}$ | 34 | 1.53 ± 0.25 $^{A}$ | 32 | 1.48 ± 0.34 $^{BC}$ |
| **Chlorides**, mmol/L | 15 | 116.66 ± 3.79 $^{ABC}$ | 29 | 106.06 ± 3.90 $^{A}$ | 34 | 108.32 ± 18.47 $^{B}$ | 32 | 118.06 ± 1.96 $^{C}$ |
| **Potassium**, mmol/L | 53 | 3.86 ± 0.68 $^{AB}$ | 29 | 4.15 ± 0.44 $^{A}$ | 34 | 3.97 ± 0.36 | 32 | 4.14 ± 0.53 $^{B}$ |
| **Sodium**, mmol/L | 15 | 147.60 ± 3.22 $^{A}$ | 29 | 160.76 ± 6.74 $^{ABC}$ | 34 | 144.06 ± 7.38 $^{BD}$ | 32 | 148.13 ± 4.00 $^{CD}$ |

$^{ABCDE}$ differences between average values rows are reliable

Degree of reliability**:** $^{A}$ p<0.01; $^{B}$ p<0.001; $^{C}$p<0.05; $^{D}$p<0.05; $^{E}$p<0.001

The levels of this macro-elements are shown in (Table . № 5 and Fig . № 4 ) . It is noteworthy that while serum calcium level moves in the average level for the dairy cows, the average phosphorus is in the lower physiological range (1.53 ± 0.25 mmol / L for the autumn and 1.48 ± 0.34 mmol / L for the winter ). During the winter months and early spring the levels have been continuously low. Once the diet is balanced in this macro element you probably have problems with the absorption or metabolism.

The altered metabolism of phosphorus is closely related to that of calcium. Calcium ions inhibit the use of phytic phosphorus and vice versa. Serum calcium showed very high levels in autumn and winter, which inhibits the absorption of phytic phosphorus and therefore although phosphorus in the ration is sufficient, it can not be absorbed completely. Calcium-phosphorus ratio in the food ration and here in physiological fluids is more important than the values to be high in both macronutrients. In our experiment a significant (r = -0.62) negative correlation between calcium and phosphorus in spring was statistically evident (Fig . № 8). Then, we registered the lowest content of calcium as well. In older animals it should be 2 : 1. In this study we found various ratios of 1.4 : 1 for spring 1.6 : 1 for summer, 2:1 for autumn and 1.96: 1 for winter.

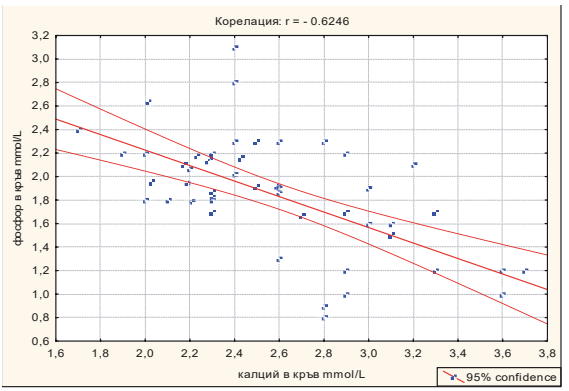



**Figure № 8.** Correlation between calcium and phosphorus in the blood in the spring

Although our research did not disclose any drastic changes in calcium and phosphorus, their proportion in the serum of cows is impaired (Fig . № 3 and Fig . № 4 ) . This abnormal ratio is most likely one of the reasons for the occurrence of reproductive disorders particularly ovarian cysts. **Plemyashov (2010)** in his research found that the basis of functional problems in reproduction of cows is due to the breach of energy, protein, vitamin and mineral metabolism, caused by unbalanced diet. He reports on excess protein levels in serum 41.8% urea at 6.3%, AST over norms - 60% and calcium-phosphorus imbalance in 69.3% of the herds. We have found lower percentage (42%) of the imbalance in the calcium and phosphorus, but the percentage of reproductive diseases is high.

### 2.2 . Analysis of potassium, sodium and chloride metabolism

The other studied macro-elements - potassium, sodium and chloride also show seasonal dynamics, not counting the direct impact on reproduction. By analyzing the levels of chlorides we found dramatic rise during the winter and the spring. At physiological rate of 95-110 mmol / L in those seasons they reached 116-118 mmol / L.

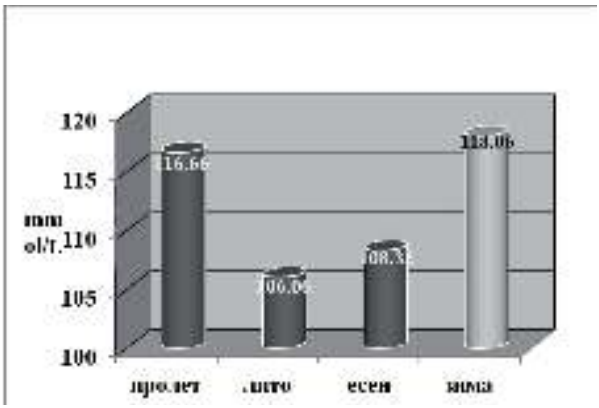



**Figure № 5**. Average serum levels of chlorides in different seasons

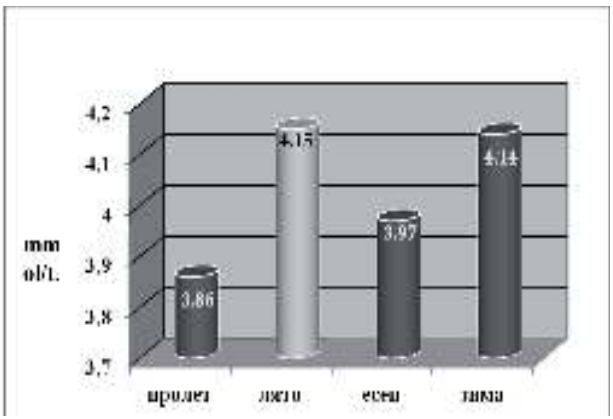



**Figure № 6**. Average serum potassium levels in different seasons

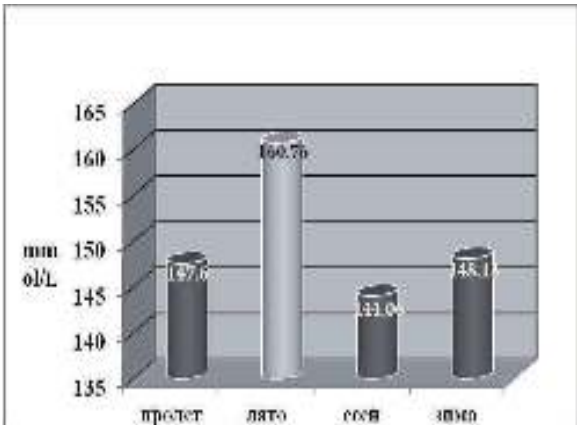



**Figure № 7**. Average serum sodium levels in different seasons

**Zhuang (2010)** in his study of the effects of the intake of common salt (Na Cl), on release of nitrogen in the urine, using the level of urea in milk as an indicator, found that urea in milk is lower when cows receive more salt in the food, while water intake and urine production are greater. The level of salt is in a positive correlation with the daily water intake (r = 0.527) and a urine production (r = 0.628), while the level of urea in milk negatively correlate with the level of salt in the diet.

***Extensive analysis in terms of macro-elements in the blood serum in connection with their participation in the intimate mechanisms of hormonal regulation of reproductive cycle and their secretion in milk.***

## 3. Biochemical analysis of the serum level of general protein, urea, AST, ALT, progesterone and blood sugar of problematic cows

Of the analyzed 148 cows with reproductive disorders there have been received and examined 331 blood samples in spring, 174 blood samples in summer, 237 blood samples in autumn and 224 in winter (**Table . № 6**).

*Serum protein*. Our studies showed that the level of general protein metabolism in cows of the farm remains constant - 83 g / L in all seasons (Table. № 6). Only, during the summer months we found significantly higher values (91.72 g / L) which compared to other months, show credible differences. The physiological range of the general protein has to move between 65 g / L and 85 g / L. Therefore, the problematic cows reached values, which

are close to the maximum, and in summer even exceed them. Similar results were reported by other researchers (**Bach et al., 2005**).

*Liver enzymes* (AST). After the analyses of 145 problematic cows (blood samples from two animals could not have been processed), it was found that the investigated liver enzymes AST are above the norm. There were normal only in winter, but reached the higher values. With reference values between 50-100 U / L in laboratory, there were registered 118.63 ± 22.66 U / L for spring, 102.16 ± 26.40 U / L for summer, 115 ± 20.38 U / L for autumn and only physiological result was found for the winter season - 97.64 ± 16.61 U / L. The activity of AST in the blood serum of the cow is in a positive correlation with the daily milk yield, milk fat, protein and lactose.

**Table № 6.** Content of urea in blood and milk, serum general protein, AST, ALT, a concentration of progesterone and blood sugar in different seasons of the year **(mean ± SD)**

| **Indicators** | **spring** | | **summer** | | **autumn** | | **winter** | |
|---|---|---|---|---|---|---|---|---|
| | **ref.** | | **ref.** | | **ref.** | | **ref.** | |
| **General protein in blood** , g/L | 53 | 83.50 ±10.67[A] | 29 | 91.72± 15.78[ABC] | 34 | 83.97± 15.08 [B] | 32 | 83.31± 18.09[C] |
| **Level of urea in blood ,** mmol/L | 53 | 5.84 ± 2.56[AB] | 29 | 6.28 ± 1.80 [C] | 34 | 7.14 ± 1.55 [AD] | 32 | 8.46 ± 2.10 [BCD] |
| **Level of ASAT**, U/L | 51 | 118.63 ± 22.66 [AB] | 29 | 102.16±26.40 [AC] | 33 | 115.00 ± 20.38 [CD] | 32 | 97.64 ± 16.61 [BD] |
| **Level of ALAT**, U/L | 53 | 45. 27 ± 12.01 [AB] | 29 | 36.45 ± 8.93[ACD] | 34 | 46.43 ± 14.11 [C] | 32 | 51.73 ± 15.57 [BD] |
| **Urea in milk**, mmol/L | 53 | 6.32 ± 1.91[ABC] | 29 | 9.74 ± 1.55[ADE] | 34 | 7.73 ± 3.24 [BD] | 32 | 7.64 ± 1.58 [I] |
| **Progesterone**, ng/ml | 53 | 1.50 ± 0.23 | 29 | 1.43 ± 0.14 | 34 | 1.52 ± 0.27 | 32 | 1.53 ± 0.14 |
| **Glucose level**, mmol/L | 15 | 5.62 ± 0.35 | | | 34 | 5.73 ± 0.46 | 32 | 5.72 ± 0.20 |

[ABCDE] differences between average values rows are reliable

Degree of reliability: [A] $p<0.05$; [B] $p<0.001$; [C] $p<0.01$; [D] $p<0.001$; [E] $p<0.001$

Similar results which relate to the activity of the liver enzyme AST in connection to the proteins and milk production are obtained by another group of researchers as well **(Dobranić et al., 2006).**

Our results are comparable with those of the other above mentioned authors. We have established the following regularity - AST activity does not increase immediately after the registration of the increased values of total serum protein, but it does the next month **(Fig. № 11 and Fig. № 12)**. The most likely cause is the compensatory physiological mechanisms. They resist the negative effects of excess toxic metabolites to a period from the beginning of calving. But then there occurred, what we have registered to be metabolic disorders. In early lactation, cows are in a negative energy balance, where functional body tissues may be metabolized in an excessive way, causing impaired function of the body.

This is a mark of liver dysfunction and affects the overall metabolism and reproductive function in cows. According to **Sangsritavong et al. (2002 )** the high levels of AST are an indicator of negative energy balance in lactating cows. The same group of researchers showed that the increased intake of protein activates a multiple hepatic circulation and which is reflected in the increased activity of the liver enzymes. Our findings confirm the same, based on our research.

In recent years, there has been an evidence (**Pande et al., 2013 ; Tanaka et al., 2013**) with absolute certainty, that all registered factors associated with liver dysfunction or presence of uterine infections have a negative effect on the follicular fluid and are manifested by a delay of the ovulation process. At the same time there was registered low level of progesterone hormone, which degrades very quickly in the liver.

*Serum urea*. The average values and standard deviation after laboratory analysis of samples from 148 cows are presented in (Table. № 6 and Fig. № 13). These analyses show significant abnormalities in serum urea in infertile cows during different seasons. In most months the values were increased and only in spring they have reached the normal values (5.84 mmol / L).

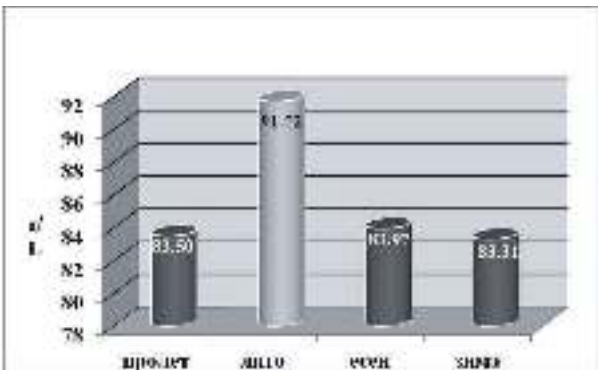



**Figure № 11**. Average serum levels of total protein in different seasons

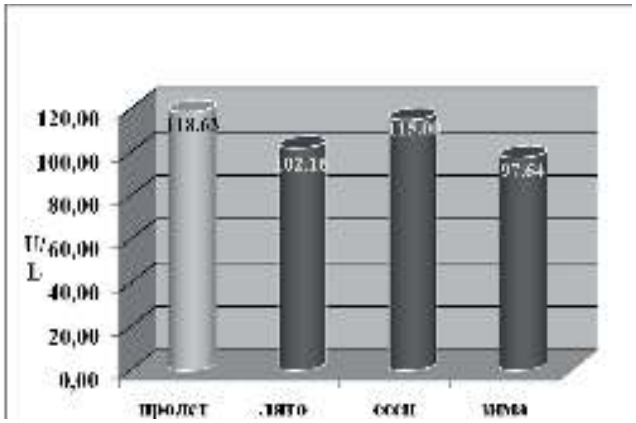



**Figure № 12**. Average serum levels of AST in different seasons

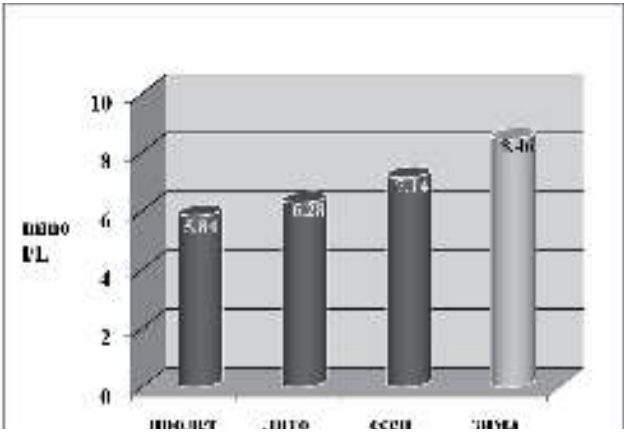



**Figure № 13**. Average serum levels of blood urea in different seasons

**Fig. № 2** shows that the lowest rates of infertility, including ovarian cysts, are registered at the same time. During the winter season, serum urea gradually rises to 8.46 mmol / L and there is the highest percentage (76.6%) of cows diagnosed with ovarian cysts. The differences of other periods are statistically proven in different degrees of reliability (Table. № 6). In our preliminary studies in Bulgaria (**Бонев ет ал., 2012**) there were presented similar patterns showing that the increasing levels of general protein in serum reflected the increased values of AST and urea in cows.

Many authors (**Moallem et al., 2011 ; Iyathurai, 2012** ) after the experiments, prove that feeding cows with high protein content during the lactation leads to an increased concentration of urea and ammonia in the bloodstream thus causing pre-ovulatory follicles and uterine cavity .

In this research we show that the highest percentage of cysts (76 % in winter) appear in cows when the values of urea in serum are the highest (**Fig . № 13**).

***Based on this research we found that one of the main causes of infertility and in particular ovarian cysts is the increased levels of blood serum urea.***

***Progesterone.*** Another factor directly involved in the complex web of causes, helping to increase or decrease fertility in cows and emitting it in milk secret is the hormone progesterone (P4). In our studies we have recorded values in the blood serum of 1.43 ± 0.14 ng / ml to 1.53 ± 0.23 ng / ml, and no statistical difference was demonstrated in different seasons. The experiments of **Дюльгер Г.П., (2008)** showed that with the formation of follicular cysts in the ovaries the concentration of progesterone in the blood throughout the cystic period did not exceed 1 ng / ml. In the transformation of the follicle or follicular cyst in lutein there was noted a transient increase in the concentration of progesterone in the blood - more than 1 ng / ml. We agree with the conclusion of the aforementioned author in which once proven progesterone was compared by ultrasound examination of cows with follicular or yellow-bodied cysts. In most cows there were found values more than 1 ng / ml. and so the diagnosis of etiopathogenesis was clearly established. We believe that once or twice determining the hormone content in blood serum is mostly indicative, in particular as regards to the type of ovarian cysts. The maximum level of progesterone in the lutein cyst formation was 3.70 ± 0.56 ng / ml, where as in the ovulatory sexual cycle it reaches to 5.6 ± 1.62 ng / ml ($p < 0.05$).

We have found in our research (Fig . № 14 and FIG . № 15) that the highest values of urea in milk (9.74 mmol / L) during the summer season coincide with the lowest levels of serum progesterone (1.43 ng / ml). Examining the level of urea in milk can indirectly lead to the conclusion that with the urea levels above 8.50 - 9.74 mmol / L the progesterone levels in blood plasma become very low, which is a major cause of infertility in cows after calving period.

To verify the accuracy of these judgments and proceeding from the previous conclusions of the veterinarians it was offered hormonal treatment of yellow-bodied cysts. The results after treatment were 52% fertility for spring season, 47% for summer, 56% for autumn and 50% for winter season.

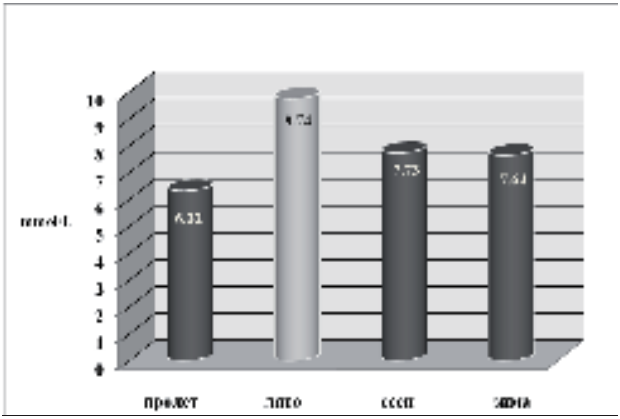


**Figure № 14**. Average level of urea in the milk during different seasons

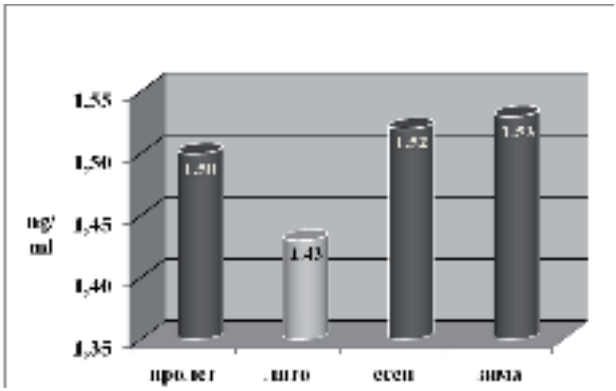


**Figure № 15**. Average serum progesterone levels during the different seasons

After having completed the clinical and laboratory tests, and detailed analysis of the results of which materials were used by many authors working in this field, we assume that the reproductive disorders in the examined farm are a major reason of the disturbed energy balance and all metabolic disorders are logical consequence and proof of this. The energy

balance of the activity of the ovarian and metabolic health status is interconnected through the transit period in dairy cows (**Butler, 2000; Galvao et al., 2010**).

## 4. The correlation between serum levels of total protein, urea, AST, ALT, progesterone, blood sugar and urea in milk secretion in problematic cows and cows with normal reproductive status (pregnant animals)

In a detailed analysis of the results obtained for the task 1 and task 2, it was found that the final metabolite that can be recorded with the available laboratory methods is the urea. The level of urea was measured both in the blood serum and in milk secretion. The presented data and analyzes confirm our preliminary hypothesis about the relationship of urea with the diagnosis of reproductive problems in dairy cows.

After determining the average values of the researched metabolites in pregnant cows after I-st insemination and problematic cows we have found parameters and regulations listed in the table. № 7. The most significant variations are those in the urea content in blood and urea in milk, while in terms of progesterone concentration and activity of AST, the differences are not statistically proven. Problematic cows were divided into two subsets with respect to the amount of urea in the serum. (more on this in MATERIAL AND METHODS)

### 4.1. Quantitative analysis of urea in blood and milk

The cows in the group "no reproductive disorders", labeled as pregnant animals, show average levels of 6.14 ± 2.05 mmol / L. Those with reproductive disorders have values 4.61 ± 0.92 mmol / L for group "A" and 8.11 ± 1.70 mmol / L for group "B".

A similar pattern was reported by other authors (Grande et al., 2009) who in the study for the monitoring of nutritional and reproductive status of dairy cows by analysing the levels of urea in milk shows that the levels of MUN below 10.0 mg / dL (4.10 mmol / L), between 13.1 mg / dL to 15.0 mg / dL and above 15.0 mg / dL (6.6 mmol / L) are a major cause of the high rate of anoestrus and low fertility after artificial insemination. Moreover these values coincide with the critical period of 55-70 days after calving. It is noteworthy that both levels which are lower than 4.61 mmol / L, and higher than 8.11 mmol / L were found in infertile cows. A consequential conclusion would be that interim blood urea levels are optimal for reproductive status.

The results show that with an increase in the level of urea in milk higher than 7.6 mmol / L or (18 mg / dL) in cows after first calving increases the number of reproductive disorders. Our results show that actually MUN values lower than 12 mg / dL and higher than 18 mg / dL had the greatest influence on fertility of dairy cows.

**Oudah (2009)** has found that concentrations of urea in milk were significantly associated with the months of monitoring the levels of urea in milk and those are the highest during the months of May (36.1 mg / dL) and September (34.2 mg / dL), and the lowest in February (29.1 mg / dL) and in March (25.2 mg / dL). In our studies we have found a minor shift in time. The highest values were measured in June and July (9.74 mmol / L) and the lowest in March and April (Table. № 6). These differences between the various experiments are insignificant and we assume that they appear due to the different geographical areas in which the experiments were conducted.

### 4.2 . Quantitative analysis of ALT and AST

Table . № 7 and Table . № 8 show that irrespectively of the effect of AST and ALT in the studied parameters, there were not statistically proven differences between groups. However, in the above cases the value of AST is always above the normal values. We have found that the activity of AST and ALT in blood serum of cows is positively correlated with daily milk yield of milk fat, protein and lactose. The values of ALT are not associated with the increase in the level of proteins and urea and are not of considerable interest in the diagnosis of reproductive disorders. Reported survey data of **Jóźwik et al. (2012)** show the influence of milkiness and stage of lactation on the activity of the liver enzymes, cholesterol and the concentration of vitamin C in the blood of dairy cows.

**Table № 7**. Contents of urea in blood and milk, concentration of progesterone and the level of AST in the blood in cows with no problems (pregnant animals) and cows with reproductive problems (mean ± SD)

| Indicators | Cows with no problems (Pregnant animals) | | Cows with reproductive disorders | | | |
|---|---|---|---|---|---|---|
| | | | Low level of urea in blood (sub-group „A“) | | High level of urea in blood (sub-gropu „B“) | |
| | ref. | | ref. | | ref. | |
| **Content of urea in blood**, mmol/L | 38 | 6.14 ± 2.05 $^{A}$ | 49 | 4.61 ± 0.92 $^{AB}$ | 61 | 8.11 ± 1.70 $^{AB}$ |
| **Content of urea in milk**, mmol/L | 38 | 7.00 ± 2.05 $^{A}$ | 49 | 6.94 ± 2.18 $^{B}$ | 61 | 8.48 ± 2.76 $^{AB}$ |
| **Progesterone concentration**, ng/ml | 38 | 1.46 ± 0.31 | 49 | 1.46 ± 0.24 | 61 | 1.47 ± 0.23 |
| **Concentration of AST**, U/L | 38 | 113.14 ± 21.74 | 49 | 109.70 ± 23.19 | 61 | 112.42 ± 22.9 |

$^{AB}$ differences between the average values in rows are reliable

$^{A}$ $p<0.05$; $^{B}$ $p<0.001$

**Table № 8.** Distribution of the studied groups of cows (n = 148) formed in relation to the indicator "low level of urea low" and "high level of urea" in blood serum (mean ± SD)

| Indicators | Low level of urea in blood (2 - 6 mmol/L) | | High level of urea in blood (above 6 mmol/L) | |
|---|---|---|---|---|
| | ref. | | ref. | |
| **Level of general protein in blood,** g/L | 59 | 84.71 ± 10.11 | 89 | 86.31 ± 15.16 |
| **Content of urea in blood,** mmol/L | 59 | 4.56 ± 0.92 [A] | 89 | 8.43 ± 1.65 [A] |
| **Level of AST**, U/L | 59 | 112.04 ± 27.52 | 89 | 110.42 ± 29.47 |
| **Level of ALT**, U/L | 59 | 41.44 ± 12.16 [A] | 89 | 47.71 ± 14.10[A] |
| **Content of urea in milk**, mmol/L | 59 | 6.92 ± 2.28 [A] | 89 | 8.12 ± 2.51[A] |
| **Progesterone concentration**, ng/ml | 59 | 1.47 ± 0.23 | 89 | 1.51 ± 0.21 |
| **Glucose level**, mmol/L | 59 | 5.88 ± 0.42 [A] | 89 | 5.66 ± 0.32 [A] |

Differences between the average values in rows are reliable -[A] $p<0.05$

Cows with low level of urea in the blood serum **(2 - 6 mmol/L) – 39.36%**
Cows with high level of urea in the blood serum **(above 6 mmol/L) – 60.64%**

### 4.3. Correlations between the studied parameters

One of the main tasks that we set to ourselves was to establish how the studied metabolites interact. The coefficients of correlation between the studied indicators are presented in Table. № 9 Table. № 10 and Table. № 11

**Table № 9.** Correlation coefficients (r) between the content of urea in blood and milk progesterone concentration and level of AST in pregnant cows

| Indicators | Urea in milk | Concentration of progesterone | Level of AST |
|---|---|---|---|
| **Urea in blood** | 0.28 | 0.05 | -0.15 |
| **Urea in milk** | | -0.16 | -0.06 |
| **Concentration of progesterone** | | | 0.11 |

**Table № 10**. Correlation coefficients (r) between the content of urea in the blood and milk and concentrations of progesterone and levels of AST in the non-pregnant cows with low content of urea in milk

| Indicators | Urea in milk | Concentration of progesterone | Level of AST |
|---|---|---|---|
| **Urea in blood** | 0.41** | 0.00 | -0.10 |
| **Urea in milk** | | 0.00 | 0.00 |
| **Concentration of progesterone** | | | 0.10 |

**Table № 11.** Correlation coefficients (r) between the content of urea in blood and milk, and concentrations of progesterone level of AST in non-pregnant cows with high levels of urea in milk

| Indicators | Urea in milk | Concentration of progesterone | Level of AST |
|---|---|---|---|
| **Urea in blood** | -0.03 | 0.16 | 0.30 ** |
| **Urea in milk** | | -0.27 ** | -0.30 ** |
| **Concentration of progesterone** | | | 0.34 ** |

** p<0.01

The levels of protein degraded in the rumen have little influence on the milkiness, but significantly affect the percentage of protein and the content of urea in milk. The concentrations of urea in plasma and milk is positively correlated (r = 0.80) **(Rafieei, 2011**). In our analysis of the problematic cows (Fig. № 17) we have found moderate positive correlation (r = 0.40) between the serum and milk urea.

The results in this direction are contradictory, as **Jankowska et al. (2010)** in their research in four consecutive lactations in Holstein Frisian cows in Poland show that the correlation coefficient between MUN, milk proteins and reproductive markers are very low.

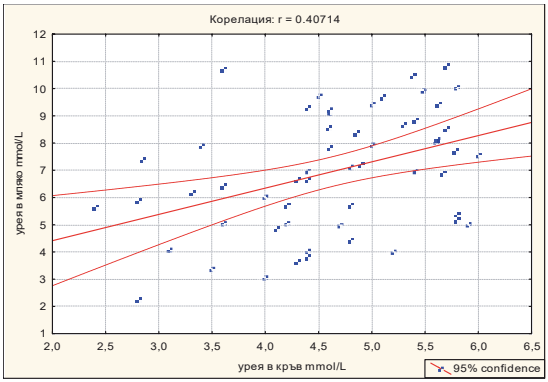



**Figure № 17.** Correlation between the level of urea in blood and urea in milk in non-pregnant cows and urea concentration in the blood below 6.00 mmol / L

## 5. LABORATORY ANALYSIS OF MILK SECRET OF COWS WITH HIGH AND NORMAL LEVELS OF UREA IN MILK

We have found in our research, that the cows with the highest level of urea in milk have demonstrated statistically higher daily milk yield ($p < 0.05$) and an extended time of coagulation ($p < 0.001$), and relatively low values of the other parameters (fat and total protein content) in comparison with the milk of the animals with the normal level of urea in milk (**Table . № 12**).

**Table № 12**. Average daily milk yield, chemical composition, physical and technological properties of milk of cows with normal level of urea in milk and cows with high levels of urea in milk

| Indicators | Normal level of urea in milk (group H) (MUN< 6.50mmol/L) | | Higher level of urea in milk (group X) (MUN> 6.50mmol/L) | |
|---|---|---|---|---|
| | mean ±SD | levels (min-max) | mean±SD | levels (min-max) |
| **Daily milk yield,** litres | 22.27 ± 3.33[A] | 18.0 - 28.9 | 25.7± 5.46[A] | 16.0 - 35.0 |
| **Fats,** % | 3.77± 0.07 | 3.7- 3.9 | 3.89± 0.32 | 3.4- 4.8 |
| **Lactose,** % | 4.45± 0.15 | 4.3- 4.7 | 4.40± 0.19 | 4.1- 4.8 |
| **Dry non-fat residue,** % | 8.33± 0.07 | 8.3- 8.4 | 8.48± 0.15 | 8.2- 8.8 |
| **General protein,** % | 3.18± 0.09 | 3.1- 3.4 | 3.42± 0.22 | 2.8- 3.7 |
| **Urea in milk, MUN,** mmol/L | 6.22± 1.40[A] | 3.1- 7.9 | 10.22± 1.73[A] | 7.4- 12.5 |
| **Number of somatic cells,** $10^3$/ml | 223.3 ± 8.15 | 210 - 230 | 225.0± 17.84 | 200 - 250 |
| **pH** | 6.66± 0.11 | 6.5- 6.8 | 6.54± 0.06 | 6.6- 6.8 |
| **°SH (**0.25 mol.L-1coNaOH**)** | 8.26± 0.82 | 7.2- 9.8 | 8.24± 1.25 | 5.8- 10.3 |
| **Initial coagulation,** min. | 10.80 ± 1.87 | 8.0- 13.0 | 10.80± 3.34 | 6.0- 20.0 |
| **Final coagulation,** min. | 49.00± 4.94[A] | 43.0-55.0 | 58.26±4.18[A] | 51.0 -65.0 |
| **Separated whey,** % | 74.94 ± 5.16[A] | 68.3 - 83.0 | 84.90± 7.99[A] | 70.0 - 94.0 |
| **pH of separated whey** | 6.52± 0.08 | 6.4- 6.6 | 6.60± 0.08 | 6.0- 6.7 |
| **°SH of separated whey** | 5.98± 0.82 | 4.5- 7.4 | 6.14± 0.97 | 4.8- 8.6 |

[A] differences between the average values indicated in columns and letters are identical with statistical accuracy ($p < 0.001$)

With regard to the amount of separated whey between groups of cows with different levels of urea in milk it was significantly different - 74.94%, respectively (cows with normal level of urea) and 84.90% (cows with high level of urea) ($p < 0.001$). According to visual evaluation of the quality and properties of the curd obtained from the cows with low levels of urea in milk it was evaluated as class I, where as that in animals with high levels of urea in milk curd was evaluated as classes III - IV (according to the classification table . № 3) In this case, the formation of whey is greater in the case of the milk with a high level of urea nitrogen, and also the production of cheese is lower due to passage of a greater amount of milk fat and casein in the whey. In the production process of cheese, higher yields per liter of milk depend on the rate of coagulation which reflects on the texture of the curd **(Chapman, 1981)**. In the rapid coagulation of the milk, the curd is deposited in a greater concentration of milk components (especially casein), which is associated with the greater hardness of the texture of the curd **(Storry et al., 1983).**

The level of urea nitrogen in milk is statistically significantly associated with the daily milk yield - in animals with high daily milk yield it was found higher concentration of urea in milk ($p < 0.05$). The same results were obtained by **Kucera (2003)**, who also establishes a positive correlation between the daily milk yield and the level of urea in milk over 10 mmol / L-1.

**Table № 13**. Coefficients of correlation between the level of urea nitrogen and chemical composition in raw milk, the coagulation properties and daily milk yield in cows with reproductive disorders

| indicators | Protein content (%) | Fats (%) | Period of initial coagulation (min) | Period of final coagulation (min) | Amount of separated whey (%) | Daily milk yield (lit.) |
|---|---|---|---|---|---|---|
| **Low level of urea in milk** | - 0.89 ** | 0.63 | - 0.59 | 0 | - 0.62 | 0.01 |
| **High level of urea in milk** | - 0.94 | 0.75 | - 0.17 | 0.07 | 0.39 | 0.48 |

** $p < 0.01$

The coefficients of correlation (Table. № 13) show that in both groups of cows there is a high negative correlation between the values of the level of urea nitrogen in the milk, and the protein content, r = -0.89 (p <0.01) and r = - 0.94. This is probably related to the incorporation of nitrogen in other alternative ways - incorporated in milk proteins or separation as urea **(Ferguson et al., 1997).** The analyses of different authors (**Rajala-Schultz et al., 2003; Roy et al., 2004; Sharma et al., 2009** ) show moderate correlation (r = 0.48) between these indicators.

**Rajala-Schultz and Saville (2003)** also found that there is a positive correlation between the urea nitrogen in milk and milk fat content in cows with different levels of urea in milk. This correlation may be associated with a large amount of neutral fibers which on the one hand increase the fat content of the milk obtained, and on the other hand, also increase the level of urea nitrogen in the milk as a result of a higher degradation of proteins in the diet. Other researches (**Klusmeyer et al., 1990**) don't show such dependency.

With regard to the relationship between the level of urea in milk, and the percentage of the separated whey of cows with a high level of urea in milk there has been established a moderate positive correlation (r = 0.39), while in the group of animals with the normal level of urea there has been a significant negative correlation (r = -0.61). This relationship is established by **Rehak et al., 2009; Kamoun et al., 2012**.

*The results obtained in the development of the thesis, allow us to confirm the original hypothesis. New generations of dairy cows, in our case of the Holstein-Frisian breed, need new types of rations with high protein content to realize their genetic traits for high milk production. When it is processed in the digestive system there are formed above-normal levels of urea, which negatively affects the entire physiological metabolism. As one of the consequences of this disorder there occurs impaired reproductive performance of cows. It is manifested clinically most often by determining ovarian follicle cysts, ovarian hypo-function and persisting of gravid yellow body over a long period after birth.*

*The main metabolites associated with impaired reproduction are high or low levels of urea in blood serum and in milk secretion and strongly increased activity of the liver enzyme AST. At the same time there occurs an increased blood circulation in the liver and rapidly peri-ovulatory degradation of progesterone and estradiol, which degrades the secretion of steroid hormones and hence reproduction. It was also found that the highest daily milk yield in cows with reproductive disorders, does not lead to a noticeable deterioration of the quality of raw milk.*

*Urea in milk directly influences the period of occurrence of the initial and final milk coagulation, as well as the kinetics and differences in the quality of the curd and the percentage of the whey produced. The results suggest that it would be interesting for cheese producers to determine the content of urea in milk at certain times of the month and the year, in order to select the correct parameters in the production of cheese.*

## CONCLUSIONS AND RECOMMENDATIONS

7.1 * Between 45 and 50 days after calving in 43.23% of the cows in the analyzed farm there were registered some proven reproductive disorders in three main groups: ovarian cysts - 60.54% , ovarian hypo-function - 30.61% and persisting yellow body - 8.84% .
7.2 Reproductive disorders in cows show clearly differentiated seasonal dynamics which is the most visibly seen in ovarian cysts 76.60% in winter and 48.28% in the summer, while the case of the ovarian hypo-function is the opposite - 51.72% in summer and 10.64% in winter.
7.3 Impaired ratio between serum levels of calcium and phosphorus is one of the reasons for the occurrence of reproductive disorders and in particular ovarian cysts.
7.4 * A second major cause of impaired reproduction, and particularly high percentage (over 76.60%) of ovarian cysts in winter is the high level of urea in the blood serum (over 8.46 mmol / L).
7.5 In case of problematic cows in terms of reproduction there were observed certain levels of serum proteins, which are close to the maximal levels (83.00 g / L), which during the summer period rise high (91.72 g/L).
7.6* AST liver enzymes in cows with reproductive disorders is above normal, with levels recorded 118.63 ± 22.66 U / L for spring, 102.16 ± 26.40 U / L for summer, 115 ± 20.38 U / L for autumn and the only physiological result that was found for the winter period - 97.64 ± 16.61 U / L. The following regularity was being established - AST activity does not increase immediately after registration of the increased values of total protein in serum, but it does in the next month.
7.7 In our analysis of the problem cows we have found a moderate positive correlation (r = 0.40) between serum and milk urea.
7.8 A cascading process that begins after an increase of the general serum protein was established in the analyzed serum and milk components. It causes high levels of urea in blood and milk, AST in serum and decrease of the progesterone hormone. The highest percentage of cysts (76% in winter) appeared in cows, when the levels of urea in serum are highest.

7.9* The highest levels of urea in milk (9.74 mmol / L) during the summer season coincided with the lowest serum progesterone (1.43 ng / ml). Thus, by exploring urea in milk we can indirectly conclude that with the urea levels above 8.50 - 9.74 mmol / L the progesterone levels in blood plasma are very low, which is a major cause of infertility in cows during the analyzed period.
7.10 The average daily milk yield of cows with a high amount of urea in milk is higher than that of cows with normal urea level (increased by 13.35% ($p < 0.001$).
7.11* In the groups of cows with normal and high concentrations of urea in milk, between the values of urea nitrogen in the milk, and the protein content there is a high negative correlation at $r = -0.89$ $p < 0.01$ for both normal $r = -0.94$ and for the high levels of urea.
7.12 On the basis of the results to the group with high levels of urea, the correlation between MUN and quantity of the separated whey at the coagulation process was moderate ($r = 0.39$), whereas with the group with normal values of urea it was found a positive correlation between MUN and the amount of the separated whey ($r = 0.61$).
7.13* There is a significant difference to the different indicators - daily milk yield, the time for initial coagulation, the level of urea in milk, time for final coagulation and the amount of separated whey between groups of cows with a high level of urea in milk and cows with a normal level of urea.
7.14 The technological quality of the curd is higher in cows with low daily milk yield.

## PRACTICE GUIDELINES

1. The values of urea in milk can serve as a prognostic indicator for the diagnosis of reproductive disorders.
2. The metabolites: calcium, phosphorus, sodium, potassium, chlorides and ALT have an indirect influence on the reproductive disorders and can not be used for metabolic profiling for the diagnosis of infertility.
3. The results obtained in terms of time of coagulation of milk and curd quality show that high levels of urea (nitrogen) in milk secretion of cows show a negative impact on the coagulation of the milk.
4. The urea in milk has a direct impact on the time of milk coagulation, the pH, the quality of the curd and the percentage of the separated whey.
5. The obtained results show that the interest of the milk production manufacturers (especially in the area of cheese production) from a practical point of view is necessary related to determining the content of urea in milk at certain times of the month and the year, in order to select the optimal parameters in the production of cheese.

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   *(IF=**2.015**)*

# “Reproductive disorders and their relationship to the metabolic profile of the Holstein - Frisian cows”


## Abstract

This research purpose, is to determine the issues related to reproductive problems, metabolic profiling and establishing relationships between dysfunction of the reproductive system and registered blood serum parameters as well among its impact on physico-chemical and technological properties of milk of the Holstein-Frisian cows. The study was made in the period of 2012 till the year 2014. To carry out the goals and purposes of the study, 148 cows of the Holstein Friesian breed were used. In the experimental group with impaired reproduction were included all cows that did not show signs of heat up to 45 days after calving. Cow’s reproductive disorders were detected by vaginal and rectal examinations, and ultrasound examination of each cow. Laboratory analysis of blood and milk samples obtained out of each season of the year, included: determination of the level of calcium, phosphorus, potassium, natrium and chlorides; serum proteins, AST, ALT, BUN - blood urea nitrogen, progesterone and glucose. Examination of the raw cow's milk samples included the following analyzes - chemical composition, MUN - milk urea nitrogen, somatic cell count, pH, titratable acidity (Soxhlet-Henkel acidity, $^{0}$SH), as well as the rennet coagulation properties. For obtaining biometric data was used statistical package "STATISTICA" v. 6.1 (StatSoft Inc., 2002).

In the study farm were discovered and observed reproductive disorders in three main groups: ovarian cysts - 60.54%, ovarian hypofunction - 30.61% and the presence of the corpus luteum persistence (8.84%). Reproductive disorders in cows indicate clearly differentiated seasonal dynamics as it was observed at ovarian cysts 76.60% in winter and 48.28% in the summer, while ovarian hypofunction is the opposite, 51.72% in summer and 10.64% in winter. Impaired ratios between serum levels of calcium and phosphorus is one of the causes of the occurrence of fertility disorders and in particular a large percentage (over 76.60%) of ovarian cysts in winter is being the high level (above 8.46 mmol / L) of blood serum urea. Liver enzyme AST in cows with reproductive disorders is above normal, while the levels recorded 118.63 ± 22.66 U / L for spring, 102.16 ± 26.40 U / L for summer, 115 ± 20.38 U / L for autumn and the only physiological result was found - 97.64 ± 16.61 U / L for the winter. In our analysis in problematic cows we found a

moderate positive correlation (r = 0.40) between serum and milk urea. The highest levels of milk urea nitrogen -MUN (9.74 mmol / L) in summer coincide with the lowest serum progesterone (1.43 ng / ml). Thereby examining the level of urea in milk, we can indirectly conclude that when BUN levels are above 8.50 - 9.74 mmol / L, the progesterone levels in plasma are very low, and this is a major cause of infertility in cow's after calving period. The average daily milk yield in cows with higher levels of urea in milk is higher compared with that of cows with normal milk urea nitrogen levels (increased by 13.35% ($p < 0.001$). In the groups of cows with normal and high concentrations of urea in milk, between the values of urea nitrogen in the milk, and the protein content there is a high negative correlation - r = - 0.89 at $p < 0.01$ for the group with normal milk urea and r = - 0.94 for the group with high levels of urea. Based on the results obtained for the group with high levels of urea, the correlation between MUN and quantity of the separated whey in the process of coagulation has been moderate (r = 0.39), whereas in the group with normal values of the milk urea was found high positive correlation between MUN an amount of the separated whey (r = 0.61). Technological quality of the curd was higher in cows with lower daily milk yield. The results suggest that it would be interesting for cheese producers to determine the content of urea in milk during certain periods of the month and the year in order to select the proper parameters in the process of cheese production.

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